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Relationship of menstrual cycle and vaginal infection in female rhesus macaques challenged with repeated, low doses of SIVmac251

Monica Morris[2], **Siddappa N. Byrareddy**[3], **Francois Villinger**[3], **Tara C. Henning**[1], **Katherine Butler**[1], **Aftab A. Ansari**[3], **Janet McNicholl**[1], and **Ellen Kersh**[1,4]

[1]Laboratory Branch, Division HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, Centers for Disease Control, Atlanta, GA, USA

[2]Total Solutions, Atlanta, GA, USA

[3]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA

Abstract

Varying susceptibility during menstrual cycling could be a factor for S(H)IV infection risk in female rhesus macaques. We retrospectively determined vaginal SIV infection time points relative to the menstrual cycle in a group of rhesus macaques (n = 11) enrolled in an HIV transmission trial. Eight of nine rhesus macaques became infected around menstruation time.

Keywords

SIV; progesterone; rhesus macaque; menstrual cycle

Introduction

Experimental vaginal S(H)IV infection of rhesus macaques can be more unpredictable than of pigtail macaques [4, 7]. This has limited the use of the species for research on HIV interventions for women. One hypothesis is that susceptibility fluctuations within menstrual cycles and irregular cycles contribute to the unpredictability. To begin to address the issue we here estimated infection time points within the menstrual cycle of rhesus macaques after they had successfully been infected in a recently completed transmission study [1].

A repeat-low dose SIVmac251 virus exposure model was used, thus mimicking the human condition where not every HIV exposure leads to infection. The model was adapted from pigtail macaque models where we have previously summarized infection time points of 43

[4]Corresponding author contact information: Ellen Kersh, 1600 Clifton Rd NE, MS A25, Bldg 17, rm 3229, Atlanta, GA 30333, ph 404 639 2728.

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SHIV-infected pigtails serving as controls in HIV prevention research studies [5, 13]. We also documented cycling in 3 additional pigtails that remained uninfected [5]. We reported that pigtail macaques have peak susceptibility to SHIV infection one week prior to and one week following menstruation [5]. This is likely due to hormonally-regulated fluctuations in immunity and the vaginal epithelium [14]. Unlike pigtails, however, wild rhesus macaque groups are seasonal breeders with mating and conceptions during the fall and winter, and births during the spring and summer, along with social hierarchies [3, 8]. Group members' ovulation is reportedly variable, and only some group members give birth, and usually only in spring [3]. It is unclear how the processes play out in captivity, and whether they affect experimental SIV acquisition. In this pilot study, we retrospectively determined menstrual cycling in relationship with SIV transmission time point in 9 infected rhesus macaques, and in 2 macaques that remained uninfected after receiving six vaginal challenges [1].

Methods

Macaques

Twelve captive, cage-housed adult female Indian rhesus macaques (*Macaca mulatta*) of Indian origin served as mock IgG-treated controls during a study testing anti-a4b7 integrin treatment [1]. They were born and housed at the Yerkes National Primate Research Center (YNPRC) of Emory University (Atlanta, GA) [1]. Animals were maintained according to the guidelines of the Institute of Laboratory Animal Resources and with approval of Emory University's IACUC [1, 9].

Virus Challenges, Hormone Analysis

Starting in February, rhesus macaques (n=12) were challenged intra-vaginally with a 1:20 dilution (5,000 $TCID_{50}$) of SIVmac251 in 1.0 ml of RPMI once weekly, resulting in infection of 10 animals after six or fewer challenges [1]. Challenges began in February and thus happened at the end of cycling season that reportedly lasts until the end of February. To determine the time point when nascent infection reached 50 copies/ml of plasma, viral loads were monitored by using bDNA quantitation on plasma aliquots [1]. Plasma was frozen at −80°C and shipped to the University of Wisconsin National Primate Research Center for progesterone analysis [10, 13]. An infected animal with ID RCd12 [1] had no samples available for analysis. Only 11 of the 12 rhesus macaque plasma samples were available to measure progesterone levels. Rising plasma progesterone was considered evidence of ovulation. We defined cycle day one as the time point post -steepest progesterone decline as previously described [13]. This allowed us to determine the menstrual cycle time point when SIV infection was first detected at greater than 50 copies/ ml of plasma.

Results

Ten animals had progesterone peaks during a total of six virus exposures (Fig. 1), while one animal RIq9 did not (Fig. 1E). All nine infected animals were cycling at time of infection. Their first positive viral load was recorded on menstrual cycle days 1, 6, 6, 8, 8, 12, 12, 14, and 27 (Fig. 2A). Two macaques remained uninfected (Fig. 1E, H); animal Rlq9 was not cycling, and cycling status of RTm11 was indeterminate. In Fig. 1, occasional measurements

during winter months preceding the virus challenges are shown to demonstrate that some animals had progesterone peaks, although sampling was too infrequent for meaningful menstrual cycle diagnosis (unconnected dots in Fig. 1). The time point of initial viremia in the nine infected macaques was found to be in the follicular phase (cycle days 1–16) of 8 macaques (95%), as compared with in the luteal phase for only 1 macaque (cycle days 17 – 32, 5%; Fig. 2A). Accounting for an estimated eclipse phase of seven days between virus transmission and first detected plasma viremia, all but one macaque became infected one week prior to or one week following menstruation (Fig. 2B). Progesterone peaks were delayed or stopped fluctuating in March and April for at least one month in all animals except for RIq9 and RTm11 (Fig. 1E, H).

Discussion

We used samples of opportunity to study whether rhesus macaques have fluctuating susceptibility to vaginal infection during menstrual cycling, as we have shown in pigtail macaques. Similar to pigtail macaques, eight of nine infected rhesus macaques preferentially became infected around menstruation time suggesting that rhesus macaques also are more susceptible to SIV following times of high progesterone [3, 8, 14]. This also confirms results by Sodora et al., who found increased virus acquisition in luteal phase when rhesus macaques were exposed in follicular or luteal phase [12]. We were limited to samples from a study in which 9 of 11 females became readily infected. It was not possible to study comprehensively why many female rhesus macaques are reportedly resistant to vaginal infection. All nine infected animals were cycling; it is possible that a lack of cycling in other parts of the year mostly accounts for the resistance. Consistent with this, one of the two resistant animals had no evidence of cycling (Fig.1E), while our second resistant animal was indeterminate at the time of virus exposure, and could not be sufficiently analyzed because very few blood samples were available (Fig. 1H).

It is possible that other factors contribute to rhesus macaques' resistance to infection. For example, rhesus macaques have considerably thicker vaginal epithelium than pigtail macaques, including greater levels of superficial vaginal keratinization throughout the menstrual cycle, and less thinning during the luteal phase [2, 4, 11].

The exact eclipse phase in each macaque was not known, because it is impossible to determine which of the repeated challenges caused infection. We therefore used seven days for an estimated eclipse phase as in previous studies [6]. It would be beneficial to analyze additional animals after year-round virus inoculations and cycle determinations in frequently collected samples. This could further elucidate the relationship between menstrual cycles and vaginal infection in captive rhesus macaques and further explore the usefulness of repeat low dose mucosal virus exposure in female rhesus macaques.

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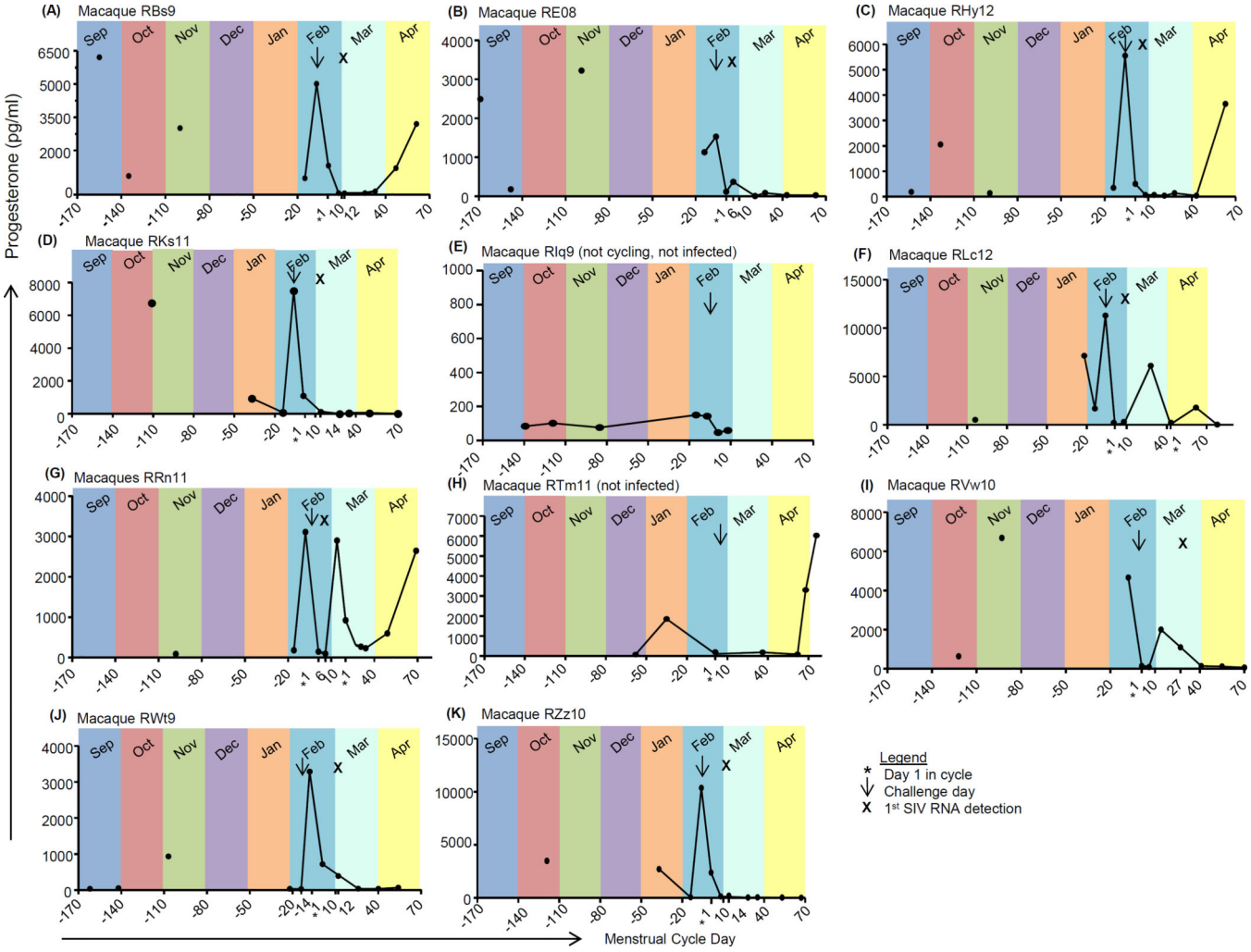

Figure 1.

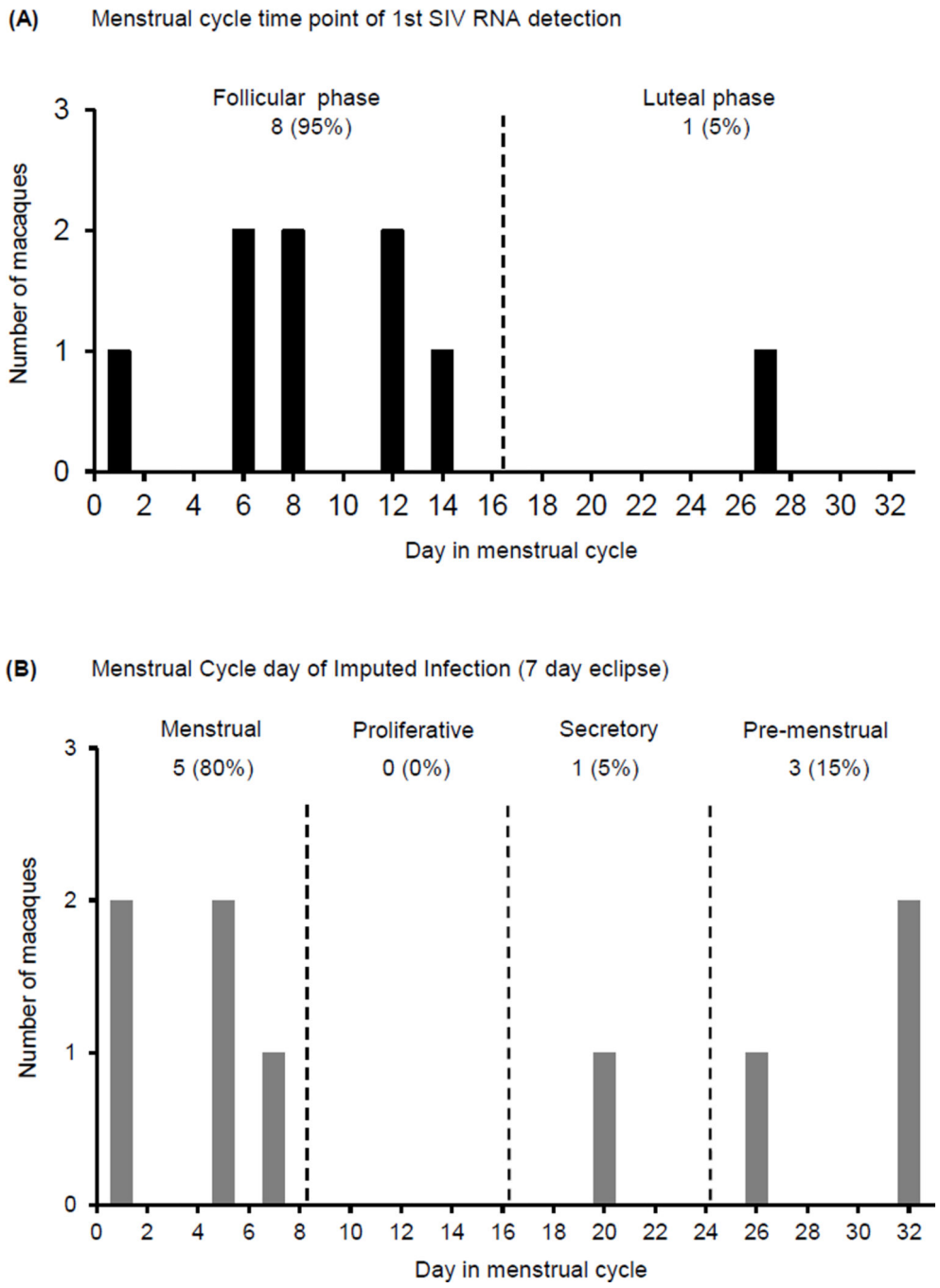

Figure 2.